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of Semi-Castration on the Sex
Ratio of the Albino Rat (*Mus
Norvegicus Albinus*)

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Reprinted from THE JOURNAL OF EXPERIMENTAL
ZoöLOGY, Vol. 10, No. 4, May 1911



May, 1911



THE EFFECTS OF SEMI-SPAYING AND OF SEMI-CASTRATION ON THE SEX RATIO OF THE ALBINO RAT (*MUS NORVEGICUS ALBINUS*.)

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The widely accepted view of a hundred years ago that the sex of an individual depends entirely upon which of the ovaries supplied the egg is generally credited to Hippocrates (460-377 B. C.). In spite of a considerable amount of adverse evidence, this theory was revived by von Seligson in 1895, and very recently it has been advocated by Dawson ('09) and by Calhoun ('10). The first two of these recent advocates of the theory are physicians, and much of the evidence that they offer in support of their views is derived from clinical cases that have come under their own observation. Calhoun's conclusions are the results of an investigation of stock breeding on a western ranch.

Medical literature contains descriptions of many cases of one-sided ovariectomy which show that eggs capable of developing into individuals of either sex are produced in each ovary. Authentic records indicate that in man, as well as in cattle, the removal of one testicle does not lead to the production of offspring of one sex only. Evidence of this kind, however, is either ignored entirely by von Seligson, Dawson and Calhoun, or its authenticity is questioned.

Among the first to make an experimental investigation of the cause of sex in mammals was Henke (1786). This investigator operated upon pigs, dogs and rabbits, removing an ovary or a testicle from each of the individuals used in the experiments. The results reported as having been obtained when these animals

were mated are very remarkable. In every instance a litter was composed of males when the left ovary and the left testicle of the parents were lacking, and entirely of females when the operation had removed the gonad from the right side of each parent. In one of his experiments Henke mated a bitch that had been sprayed on the right side with a dog that had been castrated on the left side, but no litter was produced. From the results said to have been obtained in these experiments Henke concludes that in all mammals each ovary and each testicle has its own kind of 'germ.' Eggs from the left ovary can only be fertilized with 'samen' from the left testicle, the resultant individual always being a female; conversely, male eggs from the right ovary can only be fertilized with 'samen' from the right testicle. Most modern zoölogists would not consider these conclusions warranted, since Henke made but a small number of experiments and apparently had no controls of any kind.

Ignoring the manner in which Henke carried out his experiments, von Seligson ('95a, '95b) uses the results to support his own theory, which is that of Henke expressed in more modern terms. Von Seligson himself operated upon four female rabbits, removing the left ovary from two of them and the right ovary from the remaining two: each of these rabbits was subsequently mated twice with normal males. Von Seligson states that the two females that were spayed on the left side produced males only, and that the other two rabbits had litters containing only females. The results of these experiments can hardly be considered to afford conclusive evidence in support of von Seligson's claims, since all details are wanting regarding the manner in which the experiments were conducted. Von Seligson does not mention what precautions, if any, were taken to safeguard the experiments; and in no case, apparently, did he make an autopsy to ascertain whether or not the operation had been successful.

The publication of von Seligson's theory caused a considerable amount of discussion among physicians, particularly in Germany, and a number of papers soon appeared in various medical journals giving birth records, after one-sided ovariectomy, which were not explicable according to the theory of Henke and von Selig-

son. Many of the writers of these papers stated their belief in the theory that sex is determined in the ovary, but very few of them put any faith whatever in von Seligson's contention that male eggs are segregated in the right ovary and that female eggs are produced only in the left ovary.

Goenner ('96) repeated von Seligson's experiments on rabbits, and also extended them. He removed one testicle from each of four males and one ovary from each of five females, all of the animals being about six months old when the operation was performed. From the various matings in which animals were paired that lacked the gonad on the same side of the body Goenner obtained only three litters, each of which contained both males and females. Four of the 16 young contained in these litters died before their sex was ascertained; of the remaining 12 animals, four were females and eight were males. These results indicate that von Seligson did not properly distinguish the sexes of the individuals in the various litters that he examined; but they do not give convincing evidence against his theory, since unfortunately Goenner does not state whether he killed the females and ascertained if the gonads had been entirely removed by the operation.

Dawson's revival of the right and left ovary hypothesis for man has one important modification: the spermatozoan is not considered to have any influence whatever in determining sex. According to Dawson's theory, therefore, spermatozoa from either testicle are able to fertilize eggs from either ovary. Calhoun is of the opinion that the spermatozoan may possibly have something to do with sex, and she suggests that this matter be investigated experimentally. Evidently this writer is unacquainted with much of the literature dealing with the question of sex-determination in the higher forms.

In order to test the truth of Dawson's hypothesis, Doncaster and Marshall ('10) made a small series of experiments last spring on the albino rat. One female was spayed on the right side, and a second one on the left. As soon as these rats had recovered from the effects of the operation, they were mated with normal males. The female that was spayed on the right side gave birth

to a litter containing seven young. The sex of only five of these individuals was ascertained; four of them were females, and one a male. The female lacking the left ovary produced a litter of five young, of which three were males and two were females. The breeding females were killed soon after the birth of their litters and dissected. Each was found to lack the ovary and part of the fallopian tube on the side of the body that had been operated upon. These experiments are not open to criticism on account of the manner in which they were carried out, and the results show very conclusively that, in the albino rat, eggs capable of developing into individuals of either sex can come from either ovary. Doncaster and Marshall rightly argue that because Dawson's theory is not valid for the rat is not a proof that it is also invalid for man; but they believe, as do probably most investigators, that "definite proof for another mammal detracts from its probability." Various cases in which one-sided ovariectomy in woman has been necessitated by disease have furnished evidence against the theories of von Seligson, of Dawson and of Calhoun that is fully as convincing as is that which the experiments of Doncaster and Marshall give for the albino rat.

A number of investigators, among whom may be mentioned Rauber ('00), Beard ('02), Schultze ('03) and Russo ('09), maintain that sex is determined in the ovary, although they do not believe that the male-producing eggs are segregated in the right ovary and that the female-producing eggs are all contained in the left ovary. This theory has a considerable amount of evidence in its favor, and if it be true, it is evident, as Schultze ('03) has stated, that "in der Ovogenese ist die Lösung der Geschlechtsbildung enthalten." No advocate of this theory has ventured a suggestion as to the relative distribution of the male-producing and of the female-producing eggs in each ovary, and there is the possibility that eggs of one kind may be produced in much greater numbers in one ovary than in the other. On the current hypothesis that spermatozoa are dimorphic and that the male determines sex, the possibility also exists that many more spermatozoa of one kind are produced in one testicle than in the other. If there is a constant difference in the relative distribution of the various

kinds of germ cells in the gonads, this difference should be shown by a distinct alteration of the normal sex ratio among the young produced by mating animals from which one of the gonads had been removed. To test this point a series of experiments on the albino rat (*Mus norvegicus*, *albinus*) was started in the fall of 1909. The results obtained in these experiments are given in the present paper.

The rats used in this series of experiments were operated upon by Dr. J. M. Stotsenberg of The Wistar Institute, to whom I am greatly indebted for this assistance. In all cases the operation was performed on the rats when they were 16-20 days old, the ovary or the testicle being removed while the animal was under the influence of ether. Full details regarding the manner in which the operations were made will be given in a forthcoming paper by Dr. Stotsenberg. About half an hour after the operation the young rats were returned to their nest, and they remained with their mother until they were one month old when they were fully able to care for themselves. The sexes were separated when the animals were two months old; and the rats were mated for the first time when they were about four months old. Each pair of breeding animals, earmarked for identification, occupied one of the standard cages used for the rat colony of The Wistar Institute. It was not possible, therefore, for the experiments to be invalidated by promiscuous breeding.

The sex of a newborn rat cannot be ascertained with any degree of certainty unless the animal is killed and dissected. When rats are 14-16 days old, however, the sexes are easily distinguished, as Dr. Stotsenberg has discovered, since the mammae in the females are clearly visible at this time. After this period the hair covers the entire body, and it becomes very difficult to distinguish the sexes in the living young until they are several weeks old.

Cuénot ('99) ascertained the sex of 255 young albino rats belonging to 30 different litters. He found a slightly greater number of males than of females; the sex ratio being 105.64 males to 100 females. Records that I have made of the sex of 452 young albino rats, belonging to 80 litters, give a sex ratio of 107.33

males to 100 females. Apparently, therefore, in the albino rat, as in man and various other mammals, there is normally a nearly equal proportion of the sexes among the young, although in all species there seems to be a slight excess of males.

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ALBINO RAT

Six females, belonging to two litters born in October, 1909, were operated upon when they were 16 days old. From three of these females the right ovary was removed, and from the remaining three the left ovary was taken. Two of these females, one spayed on the right side and the other spayed on the left side, never had a litter although they were paired with normal males for five months: both of these rats died of pneumonia when they were about nine months old. When dissected each female was found to have but one ovary which appeared normal in every respect. The only reason that can be suggested for the failure of these rats to breed is that they had been attacked by pneumonia when they were immature and therefore were never in a physical condition to bear young. There is evidence that rats may suffer from pneumonia, in an incipient form, for a considerable length of time with no manifestations of the disease other than a loss of weight and a failure to breed; and it is only when this disease has nearly run its course that the characteristic difficulty in breathing, which indicates the formation of pus nodules in the lung tissue, becomes at all noticeable.

Table 1 gives a summary of the number of young produced by the four semi-spayed rats when they had been mated with normal males. The letter R or L after the number given the rat indicates that the right or the left ovary had been removed.

Each litter of every female contained young of both sexes; and although there were more females than males in the total number of individuals that were produced, the excess of females was too small to be considered as significant. The two females spayed on the right side had a total of five litters which contained 22 individuals; nine of these were males and thirteen were females.

The five litters produced by the two rats that were spayed on the left side contained 25 young, of which thirteen were males and twelve were females. These results show that the sex ratio is not affected in the slightest degree by the removal of the right or of the left ovary from the breeding females, and that each ovary produces, in approximately equal numbers, eggs that are capable of developing into males and eggs that can develop into females.

Henke and von Seligson maintain that it is not possible for a male to be produced when the right gonads are lacking in the breeding pair, or for a female to develop when the left gonads have been removed. They also state that it is impossible to fertilize the eggs of an ovary with spermatozoa from the testicle on the

Table 1

| FEMALE NUMBER | NUMBER OF LITTERS | NUMBER OF YOUNG | AVERAGE NUMBER YOUNG PER LITTER | MALES | FEMALES |
|------------------|-------------------------|-----------------------|---------------------------------------|-------|---------|
| 1 (R)..... | 3 | 12 | 4.0 | 4 | 8 |
| 2 (R)..... | 2 | 10 | 5.0 | 5 | 5 |
| 3 (L)..... | 3 | 16 | 5.3 | 10 | 6 |
| 4 (L)..... | 2 | 9 | 4.5 | 3 | 6 |
| | 10 | 47 | 4.7 | 22 | 25 |

opposite side of the body. To test the truth of these hypotheses for the albino rat the following series of experiments was made:

1. Female no. 2, which had been spayed on the right side, was mated with a male from which the right testicle had been removed. A litter containing five young was obtained; three of these individuals were males and two of them were females.

2. Female no. 3, spayed on the left side, was mated with a male castrated on the left side. There was one male and one female in the resultant litter.

3. Female no. 1, lacking the right ovary, was mated with a male that had been castrated on the left side. This female had a litter containing five young, of which three were males and two were females.

4. Female no. 4, spayed on the left side, was mated with a male which lacked the right testicle. The litter produced contained six young; two of which were males and four were females.

These results show conclusively that eggs from either ovary of the albino rat can be fertilized with spermatozoa from either testicle. They also prove that males can be produced when the right gonads are lacking in the breeding animals and that females can develop when the left gonads of the breeding animals have been removed.

Table 2 gives a summary of the distribution of the sexes in all of the young produced by the four semi-spayed females.

Table 2

| FEMALE NUMBER | NUMBER OF LITTERS | NUMBER OF YOUNG | AVERAGE NUMBER YOUNG PER LITTER | MALES | FEMALES |
|------------------|-------------------------|-----------------------|---------------------------------------|-------|---------|
| 1 (R)..... | 4 | 17 | 4.1 | 7 | 10 |
| 2 (R)..... | 3 | 15 | 5.0 | 8 | 7 |
| 3 (L)..... | 4 | 18 | 4.5 | 11 | 7 |
| 4 (L)..... | 3 | 15 | 5.0 | 5 | 10 |
| | 14 | 65 | 4.64 | 31 | 34 |

The most striking fact brought out in the above table is that the litters average but 4.64 young each; a result which can doubtless be justly attributed to the removal of one of the ovaries from each of the breeding females. It is very probable that in the rat ovulation takes place in both ovaries at the same time, and that the litter of a normal female contains young that have developed from eggs derived from each ovary. Presumably, therefore, the removal of one ovary would cause a decrease in the size of the litter by lessening the number of eggs that might have been fertilized at the same time.

The average number of individuals in the 30 litters of albino rats examined by Cuénot was 8.03; while the 80 litters I have obtained contained an average of only 5.6 young. My records, however, are made up in great part from litters produced in

inbreeding experiments, and it is well known that inbreeding causes a marked decrease in the number of offspring. A normal sister of two of the semi-spayed rats was mated four times to a normal male. She had a total of 24 young, of which fourteen were males and ten were females. The average number of individuals to a litter in this instance was six. One of the females operated upon by Doncaster and Marshall gave birth to a litter of seven young; but none of the semi-spayed rats used in these experiments ever had a litter containing more than six individuals. It seems probable, therefore, from the data shown in table 2, that semi-spaying causes a decrease in the average size of the litters, although it has no appreciable effect on the sex ratio, and apparently does not decrease the number of litters a female can produce.

Two of the semi-spayed females used in these investigations died from pneumonia; the other two were etherized when it became evident that they were out of condition and would not breed again. An autopsy was made in each instance, and in no case was there any ovarian tissue on the side of the body that had been operated upon. The remaining ovary appeared normal in every female, and there was no marked increase in its size to compensate for the loss of the other ovary, as Doncaster and Marshall found to be the case in each of the two rats upon which they had operated. As these investigators operated upon adult rats and killed them about two months after the operation, it seems probable that the noticeable increase in the size of the remaining ovary must have been due to some pathological condition, and not to a normal 'compensatory hypertrophy.' There is a considerable variation in the size of the ovaries of the same rat, as well as in those of different rats; and it is not improbable that the same ovary varies in size at different times. A series of careful measurements would have to be made of a number of ovaries, removed from females at different times in the month and at different seasons of the year, in order to obtain proper standards by which to measure any apparent deviation from the normal size.

THE EFFECTS OF SEMI-CASTRATION ON THE SEX RATIO OF
THE ALBINO RAT

In February 1910, one testicle was removed from each of six males, belonging to two different litters. One male, castrated on the right side died before reaching maturity, so that two males castrated on the right side and three males castrated on the left side were available for the purposes of these experiments. Except in the cases already noted, these males were mated with normal females. The number of offspring produced, together with the distribution of the sexes in the various litters, are shown in table 3. In this table the letters R and L refer to a castration on the right or on the left side respectively.

Table 3

| MALE NUMBER | NUMBER OF LITTERS | NUMBER OF YOUNG | AVERAGE NUMBER YOUNG PER LITTER | MALES | FEMALES |
|----------------|-------------------------|-----------------------|---------------------------------------|-------|---------|
| 1 (R)..... | 4 | 17 | 4.1 | 10 | 7 |
| 2 (R)..... | 4 | 18 | 4.2 | 8 | 10 |
| 3 (L)..... | 2 | 14 | 7.0 | 9 | 5 |
| 4 (L)..... | 4 | 21 | 5.2 | 9 | 12 |
| 5 (L)..... | 3 | 13 | 4.3 | 6 | 7 |
| | 17 | 83 | 4.88 | 42 | 41 |

Practically equal proportions of the sexes were obtained as a result of this series of experiments. The two males that were castrated on the right side had a total of 35 offspring, of which 18 were males and 17 were females; the sexes were equally divided among the 48 offspring of the three males from which the left testicle had been removed. These results show that the sex ratio was not affected by the removal of the right or of the left testicle from the breeding males.

In these experiments, as table 3 shows, the average number of young in a litter was 4.88, which is very little higher than that obtained in the former experiments (table 2). The records for the litters produced by the semi-castrated males, as shown in

table 3, include the four litters obtained when these males were mated with semi-spayed females. If the records for these four litters are excluded, the remaining thirteen litters are found to contain a total of 65 individuals, which makes an average of five young to each litter. This is doubtless a low average for the litter of a normal albino rat; but it is very little lower than the average size of the litters produced in the stock colony of The Wistar Institute this past year. The small size of the litters obtained in these experiments was probably due to some external factor, and not to the castration of the breeding males.

The results obtained in these experiments indicate that, if there is a dimorphism of the spermatozoa which is associated with sex-determination, both female-producing and male-producing spermatozoa are developed in approximately equal numbers in each testicle of every normal male. A similar conclusion was recently drawn as the result of a series of investigations on the influence of the spermatozoan on the sex ratio of the toad, *Bufo lentiginosus* (King '11).

The following conclusions seem warranted by the results obtained in the series of experiments described in this paper. They are valid, at present, only for the albino rat; whether they can be extended to other mammals remains to be determined.

1. Each ovary produces eggs that are capable of developing into males and also eggs that can develop into females.

2. Each testicle contains spermatozoa that are able to fertilize the eggs from either ovary, and eggs thus fertilized develop either into males or into females.

3. The sex ratio is not altered in any way by semi-spaying or by semi-castrating the breeding animals. It follows, therefore, that:

- a. If sex is determined in the ovary, female-producing and male-producing eggs are developed in approximately equal numbers in each ovary of the normal female.

- b. If the male is responsible for sex, female-producing and male-producing spermatozoa are developed in approximately equal numbers in each testicle of the normal male.

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